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Dear Howard:

I am writing to ask whether it would be possible for you to supply me with a hopefully modest amount of some hybridization reagent useful for detection of endogenous rat leukemia virus DNA in Southern DNA transfer experiments.

The problem is as follows. Last year, while on sabbatical leave at the ICRF, I carried out an experiment (suitable only for someone on such leave) designed to ask whether retroviruses can serve as insertional mutagens. First John Wyke and I characterized an ASV-transformed rat-1 cell (called B31) which was found to carry a single ASV provirus and to revert to normal phenotype at low frequency. (Revertants constituted about 10^{-5} of the cell population and had either lost the entire provirus or undergone mutation in src. These mutants are interesting, but that is another story.) The B31 cells were then infected with a high titre stock of Moloney MuLV which I obtained from Natalie Teich (she had gotten it from Janet Hartley and passaged it only a couple of times since). The cells were grown for 1-2 weeks, to insure infection of every cell, and then put through selection procedures for the isolation of revertants.

As expected, most of the 35 or so revertants characterized appear to fall into the two classes observed spontaneously (four have no ASV provirus, over twenty have probable point mutations in src), but a few have curious lesions in the ASV provirus. In particular, at least two show clear evidence by restriction mapping of insertions of ca. $5\text{-}6\times10^{6}$Mr. In both of these cases, the insertion is present near or at the left end (3'5'-gag region) of the provirus, causing reversion by interference with the synthesis or processing of src mRNA (this has now been documented by Nancy Quintrell). In one case, the insertion is accompanied by deletion of ca. 10^{6} Mr from the left end of the provirus, including the 3'5' repeat and possibly some flanking cellular DNA; in the other case, the insertion can be mapped within two restriction sites (ca. 250 bases apart near the gag start point, but I can't say whether any deletion has occurred.

I am obviously interested in determining the nature of these inserts, but I have been confounded by several problems. First, it was apparent from the mapping data that the inserts must have restriction sites which differ from those reported for Mo-MuLV clone 1. Secondly, Inder Verma kindly supplied me with some MO-MuLV cDNA which showed that most if not all of my revertants have 1 or more copies of MuLV DNA, but again it was apparent that the maps of these proviruses do not conform with published data.

In the "interesting" revertant with an insert but no evident deletion, there are so many new MuLV proviruses that it is difficult to say whether the insert is MuLV DNA; ASV and MuLV cDNA's detect superimposible fragments produced by several enzymes, but there are enough MuLV bands to make the data less than totally convincing. I am in the process of isolating Eco RI fragments from this line for secondary digestions (and ultimately cloning in bacteria) to try to resolve the issue in this case.

The other "interesting" revertant, with an insert and a deletion, clearly has three new proviruses detectable with Inder's MuLV cDNA, but none of these seem to be the insert. Since the insert appeared after infection and is the correct size for a provirus, I certainly favor the idea that it encodes a retrovirus, but which one? An obvious possibility -- at least, with your help, a testable one -- is that the MuLV used for superinfection rescued an endogenous RaLV from the ASV-transformed rat-1 cells and that the pseudotypes formed then spread through the culture. I would very much like to test this possibility using labeled RaLV cDNA to anneal to already existing filters bearing "signature" fragments for the insertion (the insert is not cut by Xho I or Eco RI), to the isolated and secondarily digested Eco RI fragment bearing the insert (this is about to be run on the gene machine); and to the cloned insert (if and when). If you are willing to give me some help with this, it would probably be easiest if we could chat on the phone about the details (amounts, specific activities, dates, etc.). I am at 415-666-2824 at least between 9 and 6 PST almost any weekday.

I gather that Ed is now off on an English sabbatical. How is he enjoying it? Incidentally, some of your recent work was the topic of a journal club here this week---it is obviously of great interest to us.

With best regards,

Harold E. Varmus, M.D.
Professor of Microbiology & Immunology

HEV:jh